OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® HP Fungal DNA Kit

D3195-00	5 preps
D3195-01	50 preps

May 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® HP Fungal DNA Kit

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Introduction and Overview

The E.Z.N.A.® High Performance (HP) Fungal DNA Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from fresh and dried fungal tissue samples rich in polysaccharides or having lower DNA content. Up to 100 mg wet tissue (or 30 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the HiBind® matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from fungal tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

If using the E.Z.N.A.® HP Fungal DNA Kit for the first time, please read this booklet to become familiar with the procedures. This procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of Omega Bio-tek's HiBind® matrix, to purify high-quality DNA. Samples are homogenized and lysed in a high salt buffer containing CTAB and extracted with chloroform to remove polysaccharides and other components that interfere with many routine DNA isolations and downstream applications. Binding conditions are adjusted and DNA is further purified using HiBind ® DNA Mini Columns. In this way, salts, proteins, and other contaminants are removed to yield high-quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization.

New in this Edition:

- This manual has been edited for content and redesigned to enhance user readability.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D3195-00	D3195-01
Purifications	5	50
HiBind® DNA Mini Columns	5	50
2 mL Collection Tubes	10	100
CFL Buffer	5 mL	40 mL
CXD Buffer	1 mL	10 mL
DNA Wash Buffer	1.5 mL	15 mL
Elution Buffer	1.5 mL	15 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A.® HP Fungal DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in CFL Buffer and CXD Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

1. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3195-00	6 mL
D3195-01	60 mL

2. Prepare a 20 mg/mL RNase A stock solution and aliquot. Store each aliquot at -20°C and thaw before use. Each sample will require 20 µL RNase A.

Processing Fungal Samples

Choose the most appropriate protocol to follow. Procedures are described for each of dried and fresh/frozen specimens. In addition, a short protocol is given for the isolation of DNA for PCR reactions.

Dry Specimens (Page 6)	• For processing ~50 mg powdered tissue • DNA yields range from 50 µg to more than 100 µg per 100 mg dry tissue
Fresh/Frozen Specimens (Page 9)	• For processing ≤200 mg fresh or frozen tissue • Yields are similar to dry specimens
Lower DNA content samples (Page 12)	• For processing up to 200 mg dried or 450 mg fresh (or frozen) tissue • Yields are similar to dry specimens

Processing Dry Specimens

Drying allows storage of field specimens for prolonged periods of time prior to processing. Samples can be dried overnight in a 45°C oven, powdered, and stored dry at room temperature. To prepare dried samples, place ~50 mg dried tissue into a 2 mL microcentrifuge tube and grind using a pellet pestle. Disposable Kontes pestles work well and are available from Omega Bio-tek (Cat# SSI-1014-39 & SSI-1015-39). For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield.

Processing Fresh/Frozen Specimens

To prepare fresh or frozen samples, collect tissue in a 1.5 mL or 2 mL microcentrifuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable Kontes pellet pestles. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70°C for later use. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and carefully wiping the surfaces clean between samples.

E.Z.N.A.®HP Fungal DNA Kit - Protocol for Dried Samples

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 20,000 x *g*
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- Waterbath capable of 65°C
- Vortexer
- Chloroform
- Isoamyl alcohol
- Isopropanol
- 100% ethanol
- RNase A stock solution at 20 mg/mL
- Optional: 2-mercaptoethanol
- Optional: sterile deionized water

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 4
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat Elution Buffer to 65°C

1. Transfer 10-50 mg powdered dried tissue to a 2 mL microcentrifuge tube.

   Tip: Process in sets of four to six tubes: grind, add CFL Buffer and 2-mercaptoethanol, and proceed to Step 3 before starting another set. Initially, do not exceed 50 mg dried tissue. Amount can be increased according to results.

2. Add 600 µL CFL Buffer. Vortex to mix thoroughly.

   Optional: Add 10 µL 2-mercaptoethanol. Vortex to mix thoroughly. Make sure to disperse all clumps.

Optional: If necessary, add 2 µL RNase A to the lysate before Step 3 (incubation step) to remove the RNA.

3. Incubate at 65°C for 30 minutes. Invert the tube twice during incubation to mix the sample.

4. Add 600 µL chloroform:isoamyl alcohol (24:1). Vortex to mix thoroughly.

5. Centrifuge at ≥10,000 x *g* for 10 minutes.

6. Carefully aspirate 300 µL aqueous phase (top) to a new 1.5 mL microcentrifuge tube making sure not to disturb the organic phase or transfer any debris.

7. Add 150 µL CXD Buffer and 300 µL 100% ethanol. Vortex to obtain a homogeneous mixture.

   Note: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

Optional: This is the point to start the optional vacuum protocol. See Page 16 for details.

8. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes at room temperature.
3. Centrifuge at maximum speed for 20 seconds.
4. Discard the filtrate and reuse the Collection Tube.

9. Transfer the entire sample (including any precipitate that may have formed) to the HiBind® DNA Mini Column.

10. Centrifuge at 10,000 x *g* for 1 minute.

11. Discard the filtrate and the collection tube.

12. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

13. Add 650 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

14. Centrifuge at 10,000 x *g* for 1 minute.

15. Discard the filtrate and reuse the collection tube.

16. Repeat Steps 13-15 for a second DNA Wash Buffer wash step.

17. Centrifuge the empty column at maximum speed for 2 minutes.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

18. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

19. Add 100 µL Elution Buffer or sterile deionized water heated to 65°C.

    Note: Smaller volumes will significantly increase DNA concentration but result in lower yields. Using more than 200 µL for elution is not recommended.

Optional: Incubate the HiBind® DNA Mini Column at 60-70°C for 5 minutes. This step may increase yield.

20. Centrifuge at maximum speed for 1 minute.

21. Repeat Steps 18-19 for a second elution step.

    Note: This step may be performed using a new 1.5 mL microcentrifuge tube to maintain a higher DNA concentration in the first eluate.

22. Store DNA at -20°C.

E.Z.N.A.®HP Fungal DNA Kit - Protocol for Fresh/Frozen Samples

This protocol is suitable for most fresh/frozen tissue samples allowing more efficient recovery of DNA. Due to the tremendous variation in water and polysaccharide content of fungi, sample size should be limited to ≤200 mg.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 20,000 x *g*
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- Waterbath capable of 65°C
- Vortexer
- Chloroform
- Isoamyl alcohol
- Isopropanol
- 100% ethanol
- RNase A stock solution at 20 mg/mL
- Optional: 2-mercaptoethanol
- Optional: sterile deionized water

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 4
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat Elution Buffer to 65°C

1. Transfer 100 mg ground fresh/frozen tissue to a 2 mL microcentrifuge tube.

   Tip: Process in sets of four to six tubes: grind, add CFL Buffer and 2-mercaptoethanol, and proceed to Step 3 before starting another set. Initially, do not exceed 100 mg tissue. Amount can be increased up to 200 mg according to results.

2. Immediately add 500 µL CFL Buffer. Vortex to mix thoroughly.

   Optional: Add 10 µL 2-mercaptoethanol. Vortex to mix thoroughly. Make sure to disperse all clumps.

Optional: If necessary, add 2 µL RNase A to the lysate before Step 3 (incubation step) to remove the RNA.

3. Incubate at 65°C for 15 minutes. Invert the tube twice during incubation to mix the sample.

4. Add 800 µL chloroform:isoamyl alcohol (24:1). Vortex to mix thoroughly.

5. Centrifuge at ≥10,000 x *g* for 5 minutes.

6. Carefully aspirate 300 µL aqueous phase (top) to a new 1.5 mL microcentrifuge tube making sure not to disturb the organic phase or transfer any debris.

7. Add 150 µL CXD Buffer and 300 µL 100% ethanol. Vortex to obtain a homogeneous mixture.

   Note: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

Optional: This is the point to start the optional vacuum protocol. See Page 16 for details.

8. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes at room temperature.
3. Centrifuge at maximum speed for 20 seconds.
4. Discard the filtrate and reuse the Collection Tube.

9. Transfer the entire sample (including any precipitate that may have formed) to the HiBind® DNA Mini Column.

10. Centrifuge at 10,000 x *g* for 1 minute.

11. Discard the filtrate and the collection tube.

12. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

13. Add 650 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

14. Centrifuge at 10,000 x *g* for 1 minute.

15. Discard the filtrate and reuse the collection tube.

16. Repeat Steps 13-15 for a second DNA Wash Buffer wash step.

17. Centrifuge the empty column at maximum speed for 2 minutes.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

18. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

19. Add 100 µL Elution Buffer or sterile deionized water heated to 65°C.

    Note: Smaller volumes will significantly increase DNA concentration but result in lower yields. Using more than 200 µL for elution is not recommended.

Optional: Incubate the HiBind® DNA Mini Column at 60-70°C for 5 minutes. This step may increase yield.

20. Centrifuge at maximum speed for 1 minute.

21. Repeat Steps 18-19 for a second elution step.

    Note: This step may be performed using a new 1.5 mL microcentrifuge tube to maintain a higher DNA concentration in the first eluate.

22. Store DNA at -20°C.

E.Z.N.A.®HP Fungal DNA Kit - Protocol for Samples with Lower DNA Content

This modified method allows rapid isolation of DNA from fresh, frozen, or dried specimens for sample types with lower DNA content or when larger yields are essential. The procedure increases the amount of starting material so that DNA yields will generally be higher than those obtained with the previous protocols. Yields vary according to sample size and whether dried or fresh. Between 2-10 µg restrictable DNA can usually be obtained with this method.

Important: The buffer supplies with this kit is designed for standard protocols on Pages 6 and 9. Additional buffer will be required for this protocol. Buffers can be purchase separately from Omega Bio-tek, please contact Omega Bio-tek or its distributors for ordering information.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 20,000 x *g*
- Centrifuge with a swing bucket rotor capable of 3,000 x *g*
- Vortexer
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- Nuclease-free 15 mL and 50 mL centrifuge tubes
- Waterbath capable of 65°C
- Chloroform
- Isoamyl alcohol
- Isopropanol
- 100% ethanol
- RNase A stock solution at 20 mg/mL
- Optional: 2-mercaptoethanol
- Optional: sterile deionized water

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 4
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat Elution Buffer to 65°C

Note: Follow suggestions for preparation of dried or fresh samples as outlined in Sections A and B (Pages 4 and 6, respectively). Note the following limitations on sample size:

- Dry Samples - use a maximum of 200 mg ground tissue
- Fresh Samples - use a maximum of 400 mg fresh/frozen ground tissue

1. Transfer ground sample to a 15 mL centrifuge tube.

2. Add 9 mL CFL Buffer. Vortex to mix thoroughly.

   Optional: Add 10 µL 2-mercaptoethanol per 1 mL CFL Buffer. Vortex to mix thoroughly. Make sure to disperse all clumps.

3. Let sit at room temperature for 60 minutes.

4. Add 4.5 mL chloroform/isoamyl alcohol (24:1). Vortex to mix thoroughly.

5. Centrifuge at 3,000 x *g* for 10 minutes.

6. Carefully aspirate the top aqueous phase to a new 15 mL microcentrifuge tube making sure not to disturb the organic phase or transfer any debris.

7. Add 0.7 volumes isopropanol. Vortex to mix thoroughly.

8. Immediately centrifuge at 3,000 x *g* for 20 minutes. Longer centrifugation does not improve yield.

9. Carefully aspirate and discard the supernatant making sure not to dislodge the DNA pellet.

10. Place inverted centrifuge tube on a paper towel for 1 minute to allow residual liquid to drain. It is not necessary to dry the DNA pellet.

11. Add 400 µL sterile deionized water heated to 65°C. Vortex to resuspend the pellet.

    Note: A brief incubation at 65°C may be necessary to effectively dissolve the DNA.

12. Add 20 µL RNase A (20 mg/mL). Vortex to mix thoroughly.

13. Add 200 µL CXD Buffer and 400 µL 100% ethanol. Vortex to obtain a homogeneous mixture.

    Note: A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

Optional: This is the point to start the optional vacuum protocol. See Page 16 for details.

14. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes at room temperature.
3. Centrifuge at maximum speed for 20 seconds.
4. Discard the filtrate and reuse the Collection Tube.

15. Transfer 700 µL sample from Step 13 to the HiBind® DNA Mini Column.

16. Centrifuge at 10,000 x *g* for 1 minute.

17. Discard the filtrate and reuse the collection tube.

18. Repeat Steps 15-17 until all of the remaining sample (including any precipitates that may have formed) has been transferred to the HiBind® DNA Mini Column.

19. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

20. Add 650 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

21. Centrifuge at 10,000 x *g* for 1 minute.

22. Discard the filtrate and reuse the collection tube.

23. Repeat Steps 20-22 for a second DNA Wash Buffer wash step.

24. Centrifuge the empty column at maximum speed for 2 minutes.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

25. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

26. Add 100 µL Elution Buffer or sterile deionized water heated to 65°C.

    Note: Smaller volumes will significantly increase DNA concentration but result in lower yields. Using more than 200 µL for elution is not recommended.

Optional: Incubate the HiBind® DNA Mini Column at 60-70°C for 5 minutes. This step may increase yield.

27. Centrifuge at maximum speed for 1 minute.

28. Repeat Steps 26-27 for a second elution step.

    Note: This step may be performed using a new 1.5 mL microcentrifuge tube to maintain a higher DNA concentration in the first eluate.

29. Store DNA at -20°C.

E.Z.N.A.®HP Fungal DNA Kit - Vacuum Protocol

Note: Please read through previous section of this book before using this protocol.

Materials and Equipment to be Supplied by User:

- Vacuum Manifold
- Centrifuge with a swing bucket rotor capable of 3,000 x *g*
- Waterbath capable of 65°C
- 100% ethanol
- Optional: sterile deionized water

Before Starting:

- Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 4
- Prepare a mixture of chloroform: isoamyl alcohol (24:1)
- Heat Elution Buffer to 65°C

1. Complete Steps 1-7 of either the Dried or Fresh/Frozen Tissue Protocols (Pages 6 and 9, respectively).

2. Prepare the vacuum manifold according to manufacturer's instructions.

3. Connect the HiBind® DNA Mini Column to the vacuum manifold.

Optional Protocol for Column Equilibration

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Let sit for 4 minutes at room temperature.
3. Turn on the vacuum to draw the NaOH through the column.
4. Turn off the vacuum.

4. Transfer the cleared supernatant from Step 7 of the Dried or Fresh/Frozen Protocols (Pages 7 and 10, respectively) by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

5. Turn on the vacuum source to draw the sample through the column.

6. Turn off the vacuum.

7. Add 750 µL DNA Wash Buffer.

   Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

8. Turn on the vacuum source to draw the buffer through the column.

9. Turn off the vacuum.

10. Repeat Steps 7-9 for a second DNA Wash Buffer wash step.

11. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

12. Centrifuge the empty column at maximum speed for 2 minutes.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

13. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

14. Add 100 µL Elution Buffer or sterile deionized water heated to 65°C.

    Note: Smaller volumes will significantly increase DNA concentration but result in lower yields. Using more than 200 µL for elution is not recommended.

Optional: Incubate the HiBind® DNA Mini Column at 60-70°C for 5 minutes. This step may increase yield.

15. Centrifuge at maximum speed for 1 minute.

16. Repeat Steps 26-27 for a second elution step.

    Note: This step may be performed using a new 1.5 mL microcentrifuge tube to maintain a higher DNA concentration in the first eluate.

17. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged column	Debris carryover	Following extraction with chloroform: isoamyl alcohol, make sure no particulate material is transferred.
	DNA pellet not completely dissolved before applying sample to column	In the Protocol for Samples with Lower DNA Content, ensure that DNA is dissolved in water before adding CXD Buffer and ethanol. This may need repeated incubations at 65°C. Vortex during the incubations.
	Sample too viscous	Do not exceed suggested amount of starting material. Alternatively, increase the amounts of CFL and CXD Buffers and use two or more columns per sample.
Problem	Cause	Solution
Low DNA yield	Incomplete disruption of starting material	For both dry and fresh samples, obtain a fine homogeneous powder before adding CFL Buffer.
	Poor lysis of sample	Decrease the amount of starting material or increase the amount of CFL Buffer, chloroform: isoamyl alcohol, and CXD Buffer.
	DNA remains bound to column	Increase the elution volume to 200 µL and incubate the column at 65°C for 5 minutes before centrifugation.
	DNA washed off	Prepare DNA Wash Buffer according to the Preparing Reagents section on Page 4.
Problem	Cause	Solution
Problems in downstream applications	Salt carryover	DNA Wash Buffer must be at room temperature.
	Ethanol carryover	Following the second wash step, centrifuge the column for 2 minutes at maximum speed to completely dry the matrix.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
DNA Wash Buffer (100 mL)	PS010
Elution Buffer (100 mL)	PDR048
CXD Buffer (100 mL)	PD079

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PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.